complexes in the membrane. In order to better understand the folding and multimeric assembly of these proteins we have constructed a series of destabilized proteins by modifying the interface between monomers in Aquaporin Z from E. coli. We have characterized these proteins to test the the effects on the folding of the monomeric unit, the assembly of monomers into tetramers. We have also examined the consequences modified structures on the function of the water channel and the dynamics of the protein.
Structure and folding has been examined at the level of the protein topology and modifications observable by FTIR spectroscopy. These methods indicate that the surface mutations do not delectably modify the structure of the monomeric aquaporin Z. Assembly into tetramers has been investigated by hydrodynamic methods, DLS, Fluorescence anisotropy and FCS, FCCS. These methods suggest that in some mutants tetramers are not properly formed and monomeric aquaporins predominate. Some of these mutations can be suppressed by compensatory mutations on the opposite side of the interface. We report the consequences of these changes in assembly.

2525-Plat
The Role of Lipid Environment on Peptide Structure and Folding
Renee D. JiJi, Jian Xiong, Anahita Zare, Jason W. Cooley.
Chemistry, University of Missouri-Columbia, Columbia, MO, USA.
Membrane proteins are an important class of proteins that are difficult to characterize structurally and functionally. In order to gain a better understanding of the forces that govern membrane protein folding and structure, a series of simple leucine-alanine peptides ranging from 12 to 18 residues were designed. The peptides were anchored by two lysine residues at each end and spontaneously inserted into negatively charged lipid bilayers. The effects of peptide length, lipid length, bilayer/micelle composition and the presence of structure breaking residues glycine and proline on the uniformity of helical structure were evaluated. An increase in the intensities of the amide III and S bands in deep-UV resonance Raman spectra indicated loss of helical structure. Differences in peptide hydration were monitored using tryptophan fluorescence. Loss of helical structure was observed in cases of negative hydrophobic mismatch, increased peptide hydration and upon introduction helix breaking residues. No loss of helical structure was observed in cases of positive hydrophobic mismatch while the shortest peptide adopted beta-sheet structure in instances of negative hydrophobic mismatch. Greater hydration of the peptide, which occurred in surfactant/lipid micelles, magnified the helix breaking- effects of glycine and proline. These studies highlight the potential importance of the lipid environment itself on membrane protein structure.

2526-Plat
Investigation on the Interaction between Plexin Intracellular Plus Transmembrane Domains with GTPases and with the Lipid Bilayer using All-Atom Molecular Dynamics Simulations
Liqun Zhang, Buck Matthias.
Physiology and Biophysics, Case Western Reserve University, Cleveland, OH, USA.
Plexins are transmembrane receptors that receive Semaphorin guidance cues by binding them at their extracellular domains and thus are activated by them. Plexins function not only in cell migration processes, in neuronal and cardiovascular development, but also in cancer metastasis. Plexins are unique, as they are the first example of a receptor that interacts directly with small GTPases, a family of proteins that are essential for cell motility and proliferation/survival. We previously determined the structure of the Rho GTPase binding domain (RBD) of several Plexins and also of the entire intracellular regions of a Plexin-B1 [1]. Connecting the transmembrane domain to the intracellular domain of plexin, we set up a plexin-B1 all atom model bound with small Rho and Ras GTPases. The C-terminal tails of the GTPases are either farnysylated or geranylated, anchoring these proteins to the lipid membrane. Specifically, we built models of the entire intracellular plus transmembrane regions, starting from several crystal structures linked to transmembrane helices whose structure was predicted using PREDDIMER followed by microsecond-long MD refinement simulations [3]. The simulations probe interactions between the GTPases and with plexin, indicating an allosteric network that changes upon plexin-B1 binding with Rho GTPase. The models and simulations at the lipid bilayer reveal the origin of Ras and Rho specificity in plexin's function, as well as the importance of the lipid membrane in stabilizing the whole structure of plexin.
References:
[1] Hota PK, Buck M. (2012) Cell Mol Life Sci. 69(22):3765-805.
[2] Zhang L, Bouguet-Bonnet S, Buck M. (2012) Methods Mol Biol. 796:235-59.
[3] Zhang L, Polyansky A, Buck M. Submitted to PLoS Computational Biology. 2014.

2527-Plat
Role of Phospholamban Mutations in Protein-Protein Interactions
Vitaly V. Vostrikov[1], Kailey J. Soller[2], Kim N. Ha[3], Sarah E. Nelson[1], Tata Gopinath[1], Gianluigi Veglia[1].
[1]BMBB, University of Minnesota, Minneapolis, MN, USA, [2]Chemistry, University of Minnesota, Minneapolis, MN, USA, [3]Chemistry and Biochemistry, St. Catherine University, St. Paul, MN, USA.
Calcium regulation in heart muscles is achieved through a fine interplay between a variety of proteins. Of particular importance are sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) and phospholamban (PLN): SERCA transfers calcium ions against the concentration gradient and is inhibited by PLN. Inhibition is relieved upon phosphorylation of PLN at S16. Recently mutations in the *pln* gene have been linked to the progression of cardiomyopathies, raising questions about the biophysical basis of the disease.
We have investigated the behavior of the phospholamban mutants alone and in the presence of their interaction partners through complimentary techniques. Solution NMR spectroscopy provided insights into ps-ns dynamics of the regions harboring the mutation. Oriented and magic angle spinning solid-state NMR in lipid bilayers were used to probe the topology, conformation and water accessibility of phospholamban. SERCA activity assays were performed to assess the inhibitory potency of the mutants in their native or phosphorylated forms. Such multiscale approach allowed us to build a comprehensive picture of the interactions disrupted through the mutations (Vostrikov *et al*. Biochim Biophys Acta 2015).
Our data provides evidence that several essential regulatory functions are disrupted through the naturally occurring mutations. Amino acid substitutions or deletions lead to the alteration in the fold, conformation and dynamics of the regulatory domain of PLN. Such changes disrupt the PLN interactions with its binding partners, shifting the delicate balance of calcium ions transfer. We surmise that the development of cardiomyopathies elicited by the PLN mutants is linked to a variety of disrupted protein-protein interactions, rather than affecting one specific target.
This work is supported through the American Heart Association fellowships 13POST14670054 to V.V. and 13PRE16950023 to K.S.

2528-Plat
Single-Molecule FRET Detection of GXXXG-Mediated Transmembrane Helix-Helix Interactions
Yoshiaki Yano, Kotaro Kondo, Katsumi Matsuzaki.
Kyoto University, Kyoto, Japan.
Helix-helix interactions in lipid bilayers are principal processes that determine the folding, oligomerization, and conformational change of helical transmembrane proteins. Not only the amino acid sequence of the protein but also the composition of surrounding lipids significantly affect the stability of the interaction. The GXXXG motif is frequently found at interaction interface of the transmembrane region, and proposed to mediate helix associations via hydrogen bonding between Cα-H donor and the backbone C=O acceptor. However, energetic/kinetic contributions of the motif have not been well characterized.
In this study, we investigated the effect of a GXXXG-motif introduced into the center of the host transmembrane helix (AALALAA)3, examined by a single molecule FRET technique. The host helices are known to weakly self-associate in antiparallel orientations in POPC vesicles. In contrast, the GXXXG motif significantly stabilized a parallel association of the helices with lifetimes of subseconds. We also found that cholesterol suppressed the GXXXG-mediated parallel associations, demonstrating the importance of lipid environment on the helix-helix interaction

2529-Plat
Spontaneous Reconstitution of Bovine Rhodopsin into Artificial Membranes
Udeep Chawla[1], Wan Zheng[2], Liangju Kuang[2], Yunjiang Jiang[2], Suchithranga M.D.C. Perera[1], Michael F. Brown[3], Hongjun Liang[2].
[1]Departments of Chemistry and Biochemistry, University of Arizona, Tucson, AZ, USA, [2]MT, Colorado School of Mines, Golden, CO, USA, [3]Departments of Chemistry and Biochemistry, Department of Physics, University of Arizona, Tucson, AZ, USA.
Rhodopsin is a canonical G-protein-coupled receptor (GPCR) that is responsible for vision in dim light. It has the potential to serve as a high-fidelity, light-sensing molecular switch for a broad range of nanotechnologies. Previous studies revealed that the photoactivity of rhodopsin depends critically on the native lipid bilayer environment surrounding this membrane protein [1-3]. It is not yet clear how artificial membranes in synthetic systems would affect the activity of rhodopsin [4], and recent study suggests that membrane moduli may play important roles [4, 5]. Partially this uncertainty is due to the fact that it is experimentally challenging to prepare rhodopsin-supporting artificial proteomembranes with systematically varied membrane chemistry and physical properties. Here we show that bovine rhodopsin can be spontaneously reconstituted into a series of well-defined artificial membranes, including both lipid-based (i.e., liposome) and polymer-based (i.e., polymersome) membranes, via a charge-interaction-directed reconstitution